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EFFECT OF LOCALLY ISOLATED OF *LACTOBACILLUS ACIDOPHILUS* ON THE CONJUGATED LINOLEIC ACID (CLA) CONTENT IN MONTEREY LIKE CHEESE MADE FROM BUFFALO MILK

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ABSTACT

The aim of this study was to investigate the effect of locally isolated of Lactobacillus acidophilus on the conjugated linoleic acid (CLA) content In Monterey like cheese made from buffalo milk. Lactic acid bacteria were isolated from several ruminant milks using selective growth media. The isolates were identified by APL50 CHL system, and 10 isolates of Lactobacillus acidophilus were obtained. The capability of the isolates was tested in production of conjugated linoleic acid (CLA). The Lactobacillus acidophilus isolate was the most productive, for it produced 125.404 µg/ml, with a rate of % 12.54 in comparison to other isolates. Monterey like cheese samples produced from buffalo milk by cheese cultures (Lactococcus lactis sub lactis and Lactococcus lactis sub cremoris) were used as a control group while cheese cultures and Lactobacillus acidophilus were used as a mixed starter. The results were demonstrated that the moisture content of cheese was decreased with the lengthening of the repining period, and reached its maximum by 42 days. This decrease was accompanied by a rise in the salt, fat and protein percentages for the above storage period. Monterey like cheese produced with mixed starter showed an increase in the rate of soluble protein and a decrease in pH with the lengthening of the repining period mentioned above, in comparison with the cheese produced with cheese starter. The concentration of CLA was increased in Monterey like cheese during the repining period reaching its maximum in 42 days, registering a higher concentration using mixed starter in comparison with the use of cheese starter. This process was accompanied with an increase in the numbers of lactic acid bacteria. The analysis of the fat content of both kinds of cheese using GC/MS showed that it contains CLA of C9T11 with a higher rate in Monterey like cheese produced with mixed starter.

KEYWORDS: lactic Acid Bacteria, Lactobacillus Acidophilus, Monterey Cheese, CLA, Linoleic Acid

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Original Article

INTRODUCTION

CLA is a mixture of geometric and local isomers for linoleic acid C_{18}:$2C_9T_{11}$ is considered the most effectively bioactive isomer of CLA, amounting to %90 of CLA isomers. The conjugated fatty acid is available in many foods, especially the milk of ruminants (Chin *et al.*, 1992; Aydin *et al.*,2005). Several studies demonstrated the health benefits of CLA as anti-carcinogenic (Chin *et al.*, 1991) antidiabetic on diabetes type II and enhancing immune system (Houseknecht *et al.*, 1998).Cheese is considered a main source for CLA in human body. The concentration of CLA in cheese depends on its concentration in the original milk used in its production as well as production conditions and bacterial fermentations (VanNieuwwehaove *et al.*,2007). Ziatanos *et al.*(2002) showed that Greece is the largest consuming country for milk within the EU. Cases of breast cancer in Greece are the lowest among EU countries, which shows that one of the many health benefits for cheese is the protection from breast

cancer. Prandini *et al*.(2007) observed that the percentage of CLA in Italian unleavened cheese was between 6.92 – 8.11 mg/g lipid and it was 6.05-6.15 mg/g lipid in fermented Italina cheese, while it was found that the concentration of CLA in Emmental French cheese was6.8 mg/g lipid (Gnadig *et al*.,2004).Since there is a shortage of studies about CLA and its increase in the ripened cheeses like Monterey produced of buffalo milk, this study aims to raise the concentration of CLA isomer produced of buffalo milk using the locally isolated *Lactobacillus acidophilus.*

MATERIALS AND METHODS

Isolation and Identification of *Lactobacillus acidophilus*

Lactobacillus acidophilus was isolated from cow milk, goat milk and sheep milk obtained from the Centre for Agricultural Research/ College of Agriculture/ University of Basra. Buffalo milk was collected from a buffalo breeder in Garma/Basra. Pour plate method was used to isolate this organisms. Milk Samples were diluted to 10^{-1}-10^{-5}using Ringer solution (Oxoid). 1 mL of each of the diluted samples were plated into LBS Agar (Oxoid) medium, the plates were incubated at 37 °C for 72 hours under anaerobic conditions. The colonies in the plates were selected according to the specifications put forward in De Vos *et al.* (2009).The isolated bacteria were identified using colony morphology, gram stain, gas production and catalase test. The isolates lactobacilli were identified using APL_{50} medium and strips, manufactured by Biomerieux/France. Readings are added to the attached tables with the identification methods. These results are compared to other special tables and were entered to identification software apiweb TM.An inoculum (1 mL) of the mixture at the appropriate dilution was added

Producing CLA in MRS Broth Medium

The capability of local isolates to produce CLA according to the method mentioned by Rodrigeuz *et al.* (2014) was estimated using the culturing medium MRS broth, to which is added 200 µg/ml of pure CLA 99.9%supplemented by Sigma.

Extraction of Fat

Rodrigeuz *et al.* (2014) method was used in extraction of fatty acids from culturing medium.

Measuring of CLA Concentration using Spectrophotometer

CLA concentration was estimated using spectrophotometer through a scan program with a wavelength between 220-290 nm with a peak in 233 nm (Rodrigeuz *et al.*2014). CLA concentration was measured using a standard curve. The standard curve was constructed according to the method described in Zhao*et al.* (2011).

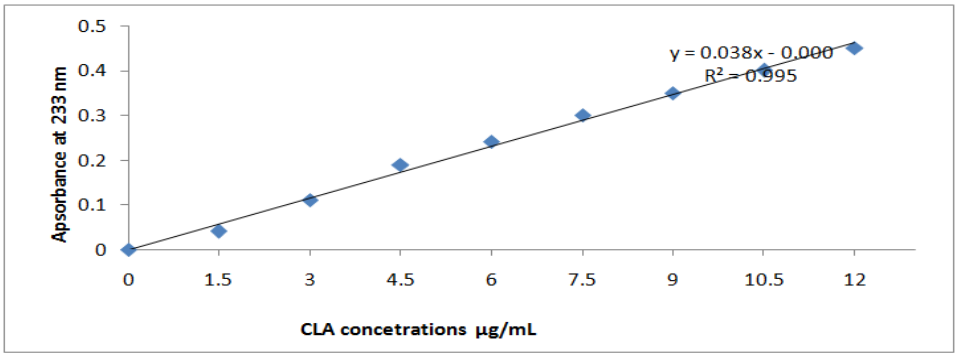

Figure 1: Standard Curve for Fatty CLA with a Wavelength 233 nm

Cheese Like Monterey Production

Monterey was made according to the method put forward by Al-abtan and Al-Durush (2010) with the addition of 200 ml/m of pure CLA 99.9% before the making of the cheese, and it was mixed well using a manual electrical mixer. The milk was pasteurized up to 72°C for 16 sec and shaken and cooled to 32° C. Cheese starter was added to produce Monterey cheese (sample A) and cheese starter with local strain *Lactobacillus acidophilus* to produce Monterey like cheese (sample B). MicrobialRennet was added according to the instructions of the supplying company. Milk was left in 32° C for 30 min and the curd was cut by $2cm^2$ and was left 5 min without shaking. The curd was then shaken gently and heated using a water bath to 37°C for 30 min until whey acidity reached %0.02. This process needed 90-95 min in buffalo milk. Part of the whey was removed and the other part was left to cover the curd. Sterilized cold water was then added, equal to one third of the whey, to clean the curd and decrease its temperature to 30° C. it was left for 5 min without shaking and then both water and whey were removed.

A rate of 2% of salt were added 3 times and mixed mildly. The curd was then placed in moulds covered with tissue, and then the curd was pressed using moderate, evenly distributed weights for 24 hrs. The piece of fabric was then removed. And the cheese was put in a sterilized and cooled incubator in 16°C and with a moisture rate of 70% for two days during which it was mildly turned and spread by hand. The cheese was then wrapped with melted paraffin wax and heated two times in 115°C for 5 sec. It was then placed in the cooled incubator in 16°C and with a moisture rate of 85% for 6 weeks. The manufacturing process was done in laminar airflow hoodsterilized with alcohol 70% and with UV light. The cooled incubator was also sterilized by alcohol 70% and chlorine 40% mg/L to eliminate any contamination. 3 liters of milk was used and the resultant curd was 450-460 g for buffalo milk. Cheese starter supplied from the Danish Chr-Hansen's was used which is composed of *Lactococcus lactissub lactis* and *Lactococcus lactissub cremoris* as well as mixed starter of cheese starter and local isolate lactobacillus acidophilus. The following tests were done for the storage periods (0, 1, 14, 28, 42) days.

Bacteriological Analyses

Cheese samples were aseptically sampled at 1, 7, 14, 28 and 42 days of ripening periods. Starter cultures (*Lactococcus lactissub lactis* and *Lactococcus lactissub cremoris*)count was determined using M17-agar of pH 7.2, aerobic incubation was carried out at 30 °C for 72 h. For enumeration of *Lactobacillus acidophilus,* the cheese samples were plated on this MRS medium then incubated at 37°C under anaerobic conditions for 72 h.

Chemical Analysis

Monterey cheese samples were chemically analyzed during ripening periods. The moisture was determined in cheese according to the Egan *et al.*(1988) method. The pH values in cheese were measured according to the Uaboi-Egbenni *et al.* (2010) method. Fat was determined by Gerber method as described by Egan *et al.*(1988).Total nitrogen and soluble nitrogen were determined according to the Uaboi-Egbenni *et al.* (2010) method. Salt in Monterey cheese was measured according to the method described in Kosikowiski (1982). The percentage of salt in cheese water was measured according to the equation:

Salt in cheese water% = percentage of salt in cheese / percentage of moisture in cheese * 100

Concentration of CLA in Cheese Samples

CLA concentration was measured using the photometrical method by scanning wavelength (220-290nm) and showed the existence of a peak in 233 nm, which indicates the existence of CLA and measuring its quantity by the standard curve.

Identifying CLA using GC/MS

Fat was extracted using the method in Coakley *et al*. (2007) and the method in Horwitz (1980) was used in fatty acid esterification. CLA isomers were identified using GC/MS as follows: Samples were diagnosed using Shimadzu Gas Chromatography Mass Spectrometry (GC/MS – PQ2010 Ultra)in GC/MS lab at the college of Agriculture/University of Basra with the following isolation conditions: Column type: 30 M * 0.25 mm i.d. DP-SMS (film thickness 0.25 m) using helium as a carrier gas with a flow rate 1 mL/sec. Injector temperature and carrier temperature was 280°C. GC oven was set on 100°C for 1 min and then it was gradually raised up to 280°C with 6° C per min. The curve spectre was set according to spectrum chart.

Statistical Analysis

Analyses of statistical differences were estimated with analysis of variance using SPSS (2012) using factorial experiments and a completely randomized design consisting of two factors: microorganism and storage periods.

RESULTS AND DISCUSSIONS

Milk is one of the most important sources for fatty acids, especially CLA. Its use as a source for isolating lactic acid bacteria might indicate the resistance these microorganism show to different concentrations of linoleic acid in the interaction by producing LA isomers, which converts Linoleic acid into conjugated linoleic acid (Jiang *et al.*, 1998; Cursoy *et al.,* 2003). These results are compatible with research that showed that ruminant milk is a rich source for lactic acid bacteria (Sieladie *et al.*, 2011; Nikita and Hemangi, 2012; Mehanne *et al.*,2013).Identification tests showed that the selected bacterial isolates of the bacillus were Gram positive, oxidase negative, catalasenegative and 10 isolates were not gas producing.

The local isolates were identified using APL_{50} CHL system on the levels of species and genus. Results of glycolysis are drawn in one attached table and entered into apiweb^{TM}. The isolates showed a compatibility of 97-99% that it belongs to the *Lactobacillus* spp genus and acidophilus species, which are obligatory homofermentative bacteria. These results are compatible with the findings of recent researchers using identification tool APL_{50} CHL. Liu *et al.* (2011) used APL_{50} CHL to identify *Lactobacilli* and *Streptococci* with a percentage of 95.6 – 98.6%. Sieledle *et al.* (2011) was identified*L. plantarum* isolated from cow milk by 99.9 % using APL_{50} CHL. Guessas *et al.* (2004) was isolated*L. acidophilus* and *L. helveticus*, as well as many obligatory and optional heterofermentative bacteria from mountain goat milk. Tabasco *et al.*(2007)was isolated *L. acidophilus* and *L. delbrueckii* subsp. *bulgaricus* from fermented milks.

Selection of Bacterial Isolates

Table 1 shows the concentration of CLA in MRS broth medium. The identified bacterial isolates showed different capabilities in producing CLA in an interaction medium of 30.288 and 125.404 µg/mLwith a percentage 3.028 and 12.540% for local isolates L.a.6 and L.a.10, respectively. This difference can be attributed to the difference in the capabilities of local isolates to produce linoleic acid isomerswhich could convert linoleic acid into the fatty CLA to remove

the poisoning content of linoleic which inhibits gram positive microorganisms, especially lactic acid bacteria due to its adsorption on bacterial cell surface and, consequently, affects the permeability of the plasma membrane(Nieman, 1954). These results also go in line with research that showed the ability of lactic acid bacteria of *Lactobacillus* sp. genus to produce different concentrationsof CLA. Xu *et al.* (2008) has mentioned that *L. acidophilus* bacteria has the ability to tolerate high concentrations of linoleic acid and to produce the fatty conjugated CLA with a percentage 65% with the use of 1 mg/ml of linoleic in MRS broth medium, whereas the isolate *L. acidophilus* ADH gave the highest converting rate of linoleic acid into CLA in MRS broth medium with the concentrations (1, 3) mg/ ml which was (26 and 65) (Xu *et al.*2006).

Table 2 shows the moisture content for cheese like Monterey produced of buffalo milk with the addition of 200 mg/ml of standard linoleic acid fertilized with 2% of cheese starter and mixed starters (cheese starter and *L. acidophilus*). The change in moisture content was examined during the ripening periods (0, 1, 14, 28, 42) days. Statistical analysis showed that there were significant effects in (P<0.01) for bacterial isolates in the percentage of the moisture content with the lengthening of the ripening period. Results showed a decrease in the moisture content with its minimum after 42 days as (42.21, 42.2, 41. 721, 41.341, 40.847) % and (42.21, 42.2, 41.545, 41.127, 40.643) %, for both starters, respectively. This difference in the moisture content during the first phases of ripening can be due to the difference in the pressing phase among different factors, as well as vaporization during the ripening period. The difference in the moisture content can be attributed to the acidity resulting of the mixed starter, which increases the solidity of the curd and thus decreases the moisture content for cheese. Besides, the high level of solid material in buffalo milk and the high rate of fats might produce a more solid curd. This result is compatible with Al-darwish and Al-abtan (2010) and Al-Sharagi *et al.* (2009) who observed a gradual fall of the Monterey cheese during the ripening periods up to 42 days. The reduction of moisture content caused significant changes in other solid material of Monterey cheese in (P<0.01) with the lengthening of the ripening period. This also led to the increase in the percentage of protein, salt, and fat for all stages of the ripening period. The highest percentage for protein was %24.88 and 23.81%, and protein rate was %30.2 and %30.4, while salt rate was 1.49 and 1.52, for both starters, respectively, as shown in Tables 2 and 3.

These results go in line with Al-darwish and Al-abtan (2010) who observed a rise in the percentages of protein, salt and fat with the lengthening of the ripening duration due to a drop in the moisture content which led to imbalance with other ingredients of the cheese, including protein. As for salt percentage, it increased with the lengthening of the ripening period and reached its maximum by day 42 with the cheese with mixed starter being higher than cheese starter. It was (3.24, 3.4%) for both starters, respectively. These starters might encourage the growth of starter bacteria, especially*L. acidophilus* which could tolerate salt concentrations mounting to %7.

Soluble nitrogen rate is an indicator for breakdown of protein (proteolysis) during the ripening period, due to its conversion into compounds of low molecular weight as well as peptides and amino acids that give cheese its suppleness, softness and special flavor (Kosikowski and Meyer 1973). Table 6 shows the percentages of soluble nitrogen for Monterey cheese produced of buffalo milk using cheese starter and mixed starter. Statistical analysis shows significant effects for the above starters in (P<0.01) for percentages of soluble nitrogen with the lengthening of the ripening period. They show a gradual rise in these percentages: (0.27, 0.327, 0.501, 0.789, 0.794)% and (0.272, 0.459, 0.624, 0.821, 0.849)% for ripening periods (0, 1, 14, 28, 42) days for both starters, respectively, with the mixed starter *L. acidophilus* recording a higher percentage of soluble nitrogen than cheese starter. This might be attributed to providing a good environment and aqueous activity, which meets the requirements for lactic acid bacteria to produce enzymes that breakdown proteins

In addition, salt percentage in cheese water made mixed starter better in proteolysis than cheese starter because the former contains *L. acidophilus* which can tolerate salt concentrations amounting to 7% (De Vos *et al.*, 2009). These results go in line with Al-Sharagi *et al.(*2009) and Al-darwish and Al.abtan (2010) who observed the rise of soluble nitrogen percentage with the lengthening of the ripening period using different isolates of *Bifidobacterium ssp*. The rise in proteolysis was attributed to the ability of these isolates to secret proteolysis enzymes.

Table 4 shows a gradual decrease in the pH with the lengthening of the ripening period, with its minimum by the end of the period as 5.41 and 5.02 for Monterey cheese produced with cheese starter and mixed starter (cheese starter and *L. accidopilus*), respectively. This was accompanied with a gradual rise of the logarithm of the number of cheese starter bacteria and*L. accidopilus*.The cheese starter (*Lactococcus lactissub lactis* and *Lactococcus lactissub cremoris*) count was increased from initial number 7.12 to 8.21Log CFU/g and from 7.12 to 8.45Log CFU/g at end of ripping period in sample A and B of cheese, respectively. *L.accidopilus* also, was increased from 7.40 to 9.12Log CFU/g at end of ripping period in sample B of cheese. This reduction might be due to the ability of lactic acid bacteria to continue producing lactic acid and other organic acids as well as its ability to consume citrates in milk causing the fall of pH (Han *et al.*, 2012). The rise of bacteria numbers with the lengthening of the ripening period might be due to the salt content and the slight fall of pH as well as the preponderance of nutrition for bacteria of amino acids, peptides and low molecular weight compounds produced by the proteolytic enzymes secreted by the starters. All these factors combined to raise the lactic acid bacteria numbers. Further, protease secretion by lactic acid bacteria caused the breakdown of caseins to smaller units like peptides and amino acids flavor compounds as well as low molecular weight compounds (Shahata and Majzoob 2005).These results are compatible with Al-Sharagi *et al. (*2009) who observed a slight fall in pH with the lengthening of the ripening period with a minimum of 5.71 during week 4. Al-darwish and Al-abtan (2010) explained this slight fall by the existence of salts and the pH as well as the metabolic products like peptides and free amino acids, which caused the logarithms of the numbers of bacteria to increase. Table 4also, shows the concentration of CLA and the lactic acid bacteria numbers in Monterey cheese produced from buffalo milk with the addition of 200µl/mL standard linoleic acid oil using cheese starters *lactococcus lactis sub cremoris* and *lactococcus lactis sub. Lactis* and mixed starter *L. acidophilus* during the ripening period (42 days). A significant rise($P<0.01$) was noticed in the concentration of CLA with the lengthening of the ripening period for both starters. It scored a maximum of (198.327 and 222.957 mg/g) after 42 days, respectively. Mixed starter was significantly higher in producing CLA in Monterey cheese than cheese starter. Construction of CLA in Monterey cheese can be attributed to the ability of starter and mixed starter bacteria in secreting isomers which converts Linoleic acid into CLA, in addition to the existence of low molecular weight compounds resulting of proteolysis by starter bacteria. These compounds are hydrogen donors, which convert linoleic acid roots into the fatty acid CLA (Darani *et al*., 2014).Lin *et al.* (1999) explained the rise of CLA in three types of Chedder (Viking, Cougar and Chedder), ripened for 3 months, to the construction of CLA via many mechanisms. One is secretion of isomerase, and the second is that ripening causes the existence of many low molecular weight compounds due to starter bacteria. These compounds are good hydrogen donors for linoleic acid rootcausing the increase of the fatty CLA in cheese. Shantha *et al.*(1992) showed that low molecular weight compounds and sodium caseinates are more active in increasing the concentration of CLA in ripened cheese. Domagala *et al.(*2013) observed that in Emmental cheese the content of CLA rises during the manufacturing phases. He showed that the concentration of CLA in milk and cheese curd during the manufacturing period is less than it is during the ripening period. The maximum production of fatty CLA was 0.64% when ripened for 6 months, and in milk it was 0.39%. He concluded that proteolysis and lipolysis have a major role in raising the concentration of CLA.

During Identifying CLA isomers in Monterey cheese by GC/MS. It was observed through figure 1 and Table 9 that CLA exists inMonterey cheese from a 1.32% after addition of cheese starters by acid area value of CLA isomer C_9T_{11}, while isomer $T_{10}C_{12}$ did not appear. While, The percentage of CLA of Monterey cheese with the addition of mixed starter was increasedto 1.80% by acid area value of the Isomers C_9T_{11} and $T_{10}C_{12}$. This might be attributed to the Linoleic acid isomerease, which converts linoleic acid into CLA. It could also be attributed to the rise of the rate of isomer C_9T_{11} in Monterey cheese produced with mixed starters. The rise of the concentration of CLA is accompanied by rise of logarithms of the product from mixed starter as well as the ability of the local isolate to secret isomerease and converting into CLA. This was proven by Kepler and Tove (1967) after they could isolate the enzyme responsible for converting Linoleic acid into CLA from *Butyrivibriofibrisolvens*. These results are compatible with Coakley *et al.*(2006) that the cheese ripened for 6 months contains CLA isomers, particularly C_9T_{11}. Buzducha and Obiedzinski (2007) observed that the cheese ripened for 8 weeks, to which are added cheese starter and mixed isolates (cheese starter + *L. acidophilus* La-5) and cheese starter + *L. casei* DN 114001) contains C_9T_{11}.

CONCLUSIONS

Probiotic bacteria *(Lactobacillusacidophilus*) can be added to improve the health-promotingpotential of different dairy products. Also, it is able to produce conjugated linoleic acid (CLA). Conjugated linoleic acid has been indicated to provide beneficial effects on health such as prevention and control of Cancer, Cardiovascular Disease, and Diabetes After screening 10 locally isolates of *Lactobacillus acidophilus*, La10 isolate was selected as a potential strain for conjugated linoleic acid production from linoleic acid in MRS broth. Cheese culture in conjunction with *Lactobacillus acidophilus* provided an effective approach to increase CLA content Monterey like cheese. Monterey like cheese produced with mixed starter demonstrated an increase in the rate of soluble protein and a decrease in pH with the lengthening of the repining period in comparison with the cheese produced with cheese starter. It was established that an edition of *Lactobacillus acidophilus* (La10) with traditional cheese culture increased the CLA content of the final Monterey like cheese. These results indicate that Monterey like cheese is a dairy product that could be used for supplying significant quantities of conjugated linoleic acid in the human food.

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APPENDICES

Table 1: Primary Selection for Local Bacterial Isolates that Produce CLA

Isolates	CLA in MRS Broth Before Inoculation	CLA µg/mL	CLA%
L. a.1	Non	51.274	5.127
L. a.2	Non	48.440	4.844
L. a.3	Non	61.544	6.154
L. a.4	Non	110.113	11.011
L. a.5	Non	101.813	10.181
L. a.6	Non	30.288	3.028
L. a.7	Non	48.101	4.810

Table 1: Contd.,			
L. a.8	Non	62.891	6.289
L. a.9	Non	74.062	7.406
L. a.10	Non	125.404	12.540

Table 2: Percentages for Moisture, Protein, Soluble Nitrogen and Fat in Monterey Cheese Samples Produced with Cheese Starter (Sample A) and Mixed Starter (Sample B)

The ripening period (day)		Moisture%	Protein%	Soluble nitrogen%	Fat %
0	Sample A	42.21	22.38	0.220	35.2
	Sample B	42.21	22.38	0.220	35.2
1	Sample A	42.20	22.55	0.227	35.4
	Sample B	42.20	22.48	0.270	35.5
14	Sample A	41.72	22.86	0.413	35.5
	Sample B	41.55	22.88	0.524	35.6
28	Sample A	41.34	23.22	0.619	35.8
	Sample B	41.13	23.37	0.721	35.8
42	Sample A	40.85	23.32	0.751	36.2
	Sample B	40.64	23.41	0.844	36.4

Table 3: Percentages for Salts in Monterey Cheese Samples Produced with Cheese Starter (Sample A) and Mixed Starter (Sample B)

The Ripening Period (Day)	Monterey Cheese (A)	Monterey Cheese (B)	Salt in Cheese Water (A)	Salt in Cheese Water (B)
0	1.21	1.22	2.86	2.81
1	1.23	1.29	2.93	3.04
14	1.27	1.23	3.06	3.2
28	1.32	1.27	3.22	3.36
42	1.36	1.32	3.24	3.40

Table 4: Concentration of CLA, pH and Microbial Counts (log_{10}CFU/g) for the Starters used in Monterey Cheese Samples Produced with Cheese Starter (Sample A) and Mixed Starter (Sample B)

The ripening period (day)		CLA mg/g	pH	Log CFU/g Cheese starter	Log CFU/g *L. acidophilus*
0	Sample A	134.142	5.90	7.12	0
	Sample B	134.142	5.60	7.12	7.40
1	Sample A	156.136	5.80	7.54	0
	Sample B	175.631	5.51	7.49	7.86
14	Sample A	172.791	5.71	7.74	0
	Sample B	199.187	5.42	7.81	8.22
28	Sample A	181.327	5.64	8.13	0
	Sample B	210.837	5.24	8.09	8.37
42	Sample A	198.327	5.41	8.21	0
	Sample B	222.957	5.02	8.45	9.12

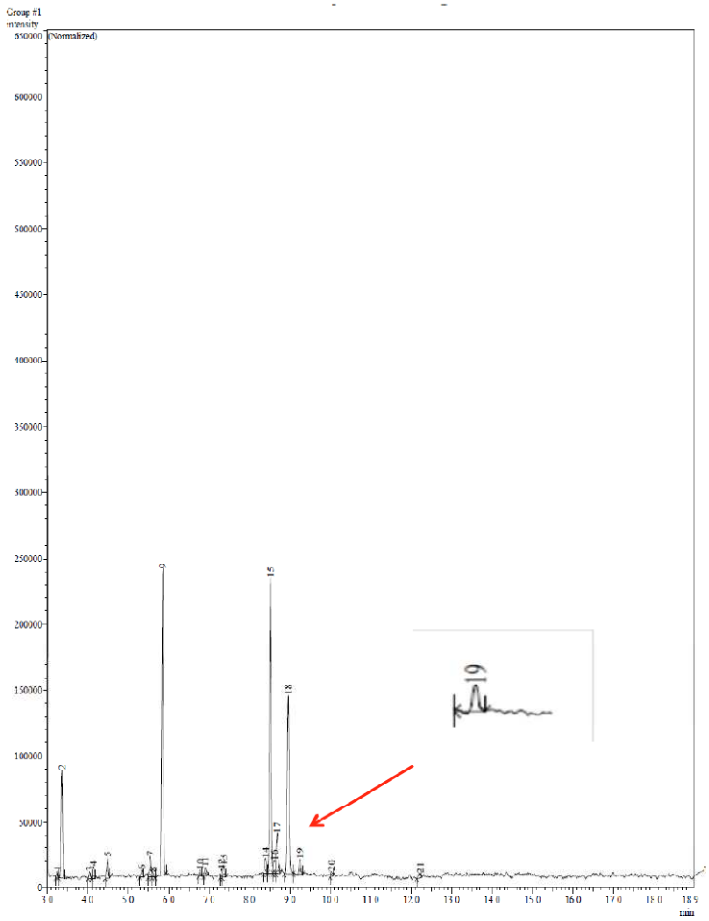

Figure 2: Fatty Acids and Conjugated Linoleic Acid isomers in Monterey Cheese Sample A

Table 5: Fatty Acids and Conjugated Linoleic Acid isomers in Monterey Cheese Sample A

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name
1	3.251	13121	0.54	5522	0.66	Heneicosyl pentafluoropropionate
2	3.564	189207	7.86	81474	9.80	Methyl tetradecanoate
3	4.048	13393	0.56	5374	0.65	Pentadecanoic acid, methyl ester
4	4.143	23816	0.99	9074	1.09	Tridecanoic acid, 12-methyl-, methyl ester
5	4.493	45605	1.89	15699	1.89	Pentadecanoic acid, methyl ester
6	5.332	15256	0.63	5461	0.66	Octacosyl trifluoroacetate
7	5.547	48607	2.02	15700	1.89	9-Hexadecenoic acid, methyl ester, (Z)-
8	5.617	3643	0.36	3939	0.47	Z,Z-6,25-Tetratriacontadien-2-one
9	5.855	637289	26.46	232428	27.95	Hexadecanoic acid, methyl ester
10	6.778	11678	0.48	4612	0.55	Heptadecanoic acid, methyl ester
11	6.906	19660	0.82	6688	0.80	Silane, [[(3 beta.)-gorgost-5-en-3-yl]oxy]tri
12	7.308	15820	0.66	5999	0.72	Hexadecanoic acid, tetradecyl ester
13	7.357	25394	1.05	8616	1.04	Heptadecanoic acid, methyl ester
14	8.397	34060	1.41	11418	1.37	9,12-Octadecadienoic acid (Z,Z)-, methyl es
15	8.521	692716	28.76	224600	27.01	8-Octadecenoic acid, methyl ester
16	8.617	30498	1.27	9937	1.19	Cholest-2-eno[2,3-b]quinoxaline, 6'-nitro-
17	8.685	105634	4.39	30878	3.71	9-Octadecenoic acid (Z)-, methyl ester
18	8.956	419717	17.43	135423	16.28	Octadecanoic acid, methyl ester
19	9.248	31752	1.32	11142	1.34	Methyl 9-cis,11-trans-octadecadienoate
20	10.027	10391	0.43	4156	0.50	Z,Z-6,24-Tritriacontadien-2-one
21	12.222	16083	0.67	3444	0.41	N-Acetyl-N-methyl-3-aminocholestane (3.1
		2408340	100.00	831584	100.00	

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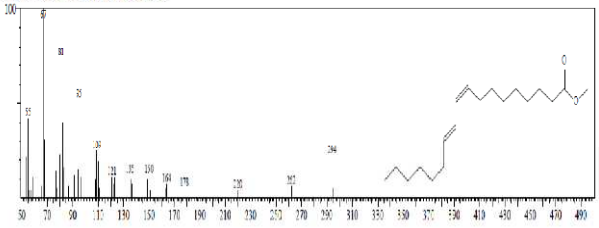

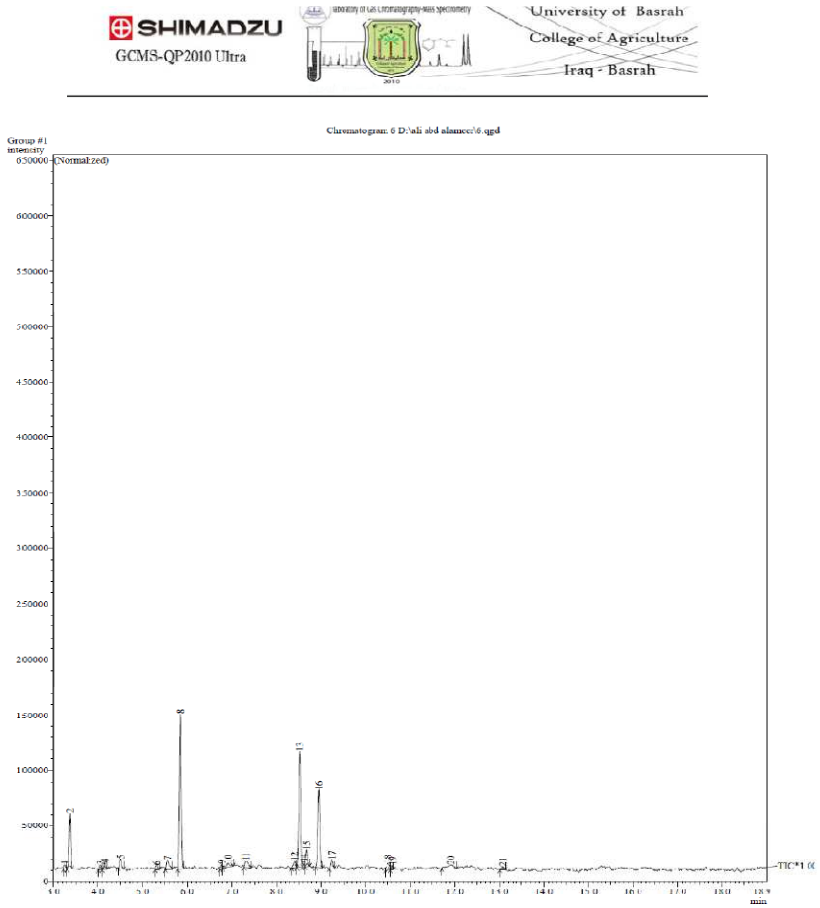

Figure 3: Fatty Acids and Conjugated Linoleic Acid isomers in Monterey like Cheese Sample B

Table 6: Fatty Acids and Conjugated Linoleic Acid isomers in Monterey like Cheese Sample B

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name
1	3.249	12570	0.89	4075	0.87	Oleyl alcohol, heptafluorobutyrate
2	3.366	115289	8.14	50564	10.85	Methyl tetradecanoate
3	4.052	12548	0.89	4284	0.92	2-Dotriacontanone
4	4.150	15617	1.10	5959	1.28	Silane, [3,5-dichloro-1-(1-chloroheptyl)-1,2
5	4.497	25203	1.78	8659	1.86	Pentadecanoic acid, methyl ester
6	5.312	14024	0.99	3897	0.86	Triacontanoic acid, methyl ester
7	5.549	38741	2.73	8543	1.83	10-Acetoxy-2-hydroxy-1,2,6a,6b,9,9,12a-he
8	5.852	383718	27.09	140399	30.13	Hexadecanoic acid, methyl ester
9	6.778	9556	0.67	3794	0.81	Ergostane-5,25-diol, 3,6,12-tris[(trimethylsil
10	6.919	23180	1.64	3568	0.77	Triacontane, 1,30-dibromo-
11	7.306	34161	2.41	6032	1.29	Isopropyl Palmitate
12	8.399	22049	1.56	6319	1.36	Methyl 5,9,23-tricosatrienoate
13	8.517	316172	22.32	104925	22.52	9-Octadecenoic acid (Z)-, methyl ester
14	8.617	15128	1.07	4344	0.93	9,19-Cyclolanost-25-en-3-ol, 24-methyl-, (3
15	8.685	43077	3.04	15254	3.27	9-Octadecenoic acid (Z)-, methyl ester
16	8.955	219589	15.51	70396	15.11	Octadecanoic acid, methyl ester
17	9.241	25503	1.80	8217	1.76	Methyl 9-cis,11-trans-octadecadienoate
18	10.498	31272	2.21	5611	1.20	3-[3-Bromophenyl]-7-chloro-3,4-dihydro-2
19	10.608	10878	0.77	3790	0.81	7-Chloro-5-phenyl-1-[4-(4-phenyl-piperazin
20	11.909	38296	2.70	3916	0.84	2-[2-(Acridin-9-ylamino)-3-methyl-butyryl a
21	13.069	9761	0.69	3292	0.71	Phenol, 4,6-dichloro-2-(4-iodophenylimino
		1416532	100.00	465936	100.00